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Tenofovir Gel for the Prevention of Herpes Simplex Virus Type 2 Infection

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Abstract

BACKGROUND—Globally, herpes simplex virus type 2 (HSV-2) infection is the most common cause of genital ulcer disease. Effective prevention strategies for HSV-2 infection are needed to achieve the goals of the World Health Organization global strategy for the prevention and control of sexually transmitted infections.

METHODS—We assessed the effectiveness of pericoital tenofovir gel, an antiviral microbicide, in preventing HSV-2 acquisition in a subgroup of 422 HSV-2–negative women enrolled in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 study, a double-blind, randomized, placebo-controlled trial. Incident HSV-2 cases were identified by evidence of seroconversion on an HSV-2 IgG enzyme-linked immunosorbent assay between study enrollment and exit. A confirmatory analysis was performed by Western blot testing.

RESULTS—The HSV-2 incidence rate was 10.2 cases per 100 person-years (95% confidence interval [CI], 6.8 to 14.7) among 202 women assigned to tenofovir gel, as compared with 21.0 cases per 100 person-years (95% CI, 16.0 to 27.2) among 222 women assigned to placebo gel (incidence rate ratio, 0.49; 95% CI, 0.30 to 0.77; $P = 0.003$). The HSV-2 incidence rate among the 25 women with vaginal tenofovir concentrations of 10,000 ng per milliliter or more was 5.7 cases per 100 person-years, as compared with 15.5 cases per 100 person-years among the 103 women with no detectable vaginal tenofovir (incidence rate ratio, 0.37; 95% CI, 0.04 to 1.51; $P = 0.14$). As confirmed by Western blot testing, there were 16 HSV-2 seroconversions among women

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assigned to tenofovir gel as compared with 36 among those assigned to the placebo gel (incidence rate ratio, 0.45; 95% CI, 0.23 to 0.82; $P = 0.005$).

CONCLUSIONS—In this study in South Africa, pericoital application of tenofovir gel reduced HSV-2 acquisition in women. (Funded by the U.S. Agency for International Development and others; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00441298) number, NCT00441298.)

Globally, Herpes Simplex Virus Type 2 (HSV-2) infection is among the most common sexually transmitted infections and is the leading cause of genital ulcers. Available global estimates indicate that in 2012 approximately 417 million sexually active adults 15 to 49 years of age had an existing prevalent HSV-2 infection.¹ A U.S. survey covering the period from 1999 through 2010 showed a 15.7% prevalence of HSV-2 among persons 14 to 49 years of age.² Sub-Saharan Africa is the most severely affected region of the world; up to 80% of sexually active women and up to 50% of sexually active men are infected with HSV-2.^{3–5} Effective prevention strategies for HSV-2 infection are needed to achieve the goals of the World Health Organization global strategy for the prevention and control of sexually transmitted infections.⁵

Tenofovir, a nucleotide reverse-transcriptase inhibitor, is widely used in its oral formulation for the treatment of human immunodeficiency virus (HIV) infection. Pericoital application of a topical vaginal-gel formulation of tenofovir was shown in one study to reduce HIV acquisition.⁶ Subsequent trials of daily application,⁷ as well as pericoital application,⁸ of tenofovir gel showed no significant protection against HIV infection, most likely owing to low rates of adherence among women in these studies.

Topical application is associated with vaginal tenofovir concentrations that are up to 1000 times as high as those achieved with oral tenofovir,^{9,10} reaching levels well above the half-maximal effective concentration (EC_{50}) for HSV-2.¹¹ This study therefore assessed whether pericoital application of tenofovir gel is effective in reducing HSV-2 acquisition in women.

Methods

Caprisa 004 Study Design

HSV-2-seronegative women who were enrolled in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial comprised the population of this study. The overall CAPRISA 004 study, which was conducted from 2007 to 2010, was a double-blind, placebo-controlled, randomized trial that was designed to assess whether tenofovir gel prevents HIV infection in women.

In the CAPRISA 004 trial, 889 eligible HIV-negative urban and rural South African women were randomly assigned to receive either tenofovir gel or placebo gel. Tenofovir gel consisted of approximately 40 mg of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) monohydrate in a solution of purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, and hydroxyethylcellulose. Gilead Sciences donated PMPA monohydrate for the manufacture of tenofovir gel; the company did not play any additional role in the study or have access to the data presented here. By arrangement with Gilead Sciences and CONRAD (a nonprofit reproductive health organization), the biotechnology

agency of the South African government received a voluntary, nonexclusive, royalty-free license for tenofovir gel for local manufacture and low-cost distribution in Africa. The placebo was the universal microbicide placebo, hydroxyethylcellulose gel, for which there is no evidence in animal models of either a protective or susceptibility-enhancing effect with regard to HSV-2.¹²

Randomization was performed in random permuted blocks of 6 or 12 and was stratified according to site. The randomization procedure was conducted by an independent statistician who issued study-drug assignments by letters in sealed, opaque envelopes, which were stored securely; each envelope was opened in sequence by the study pharmacist once the study clinician had enrolled the participant. Participants' HSV-2 serologic status was not known during the process of enrollment and randomization, as well as during follow-up in the trial (as detailed below, HSV-2 serologic status was determined retrospectively).

Tenofovir and placebo gels appeared identical and were dispensed in the same prefilled vaginal applicators with identical packaging. Women were instructed to insert one dose of the gel within 12 hours before sex, a second dose as soon as possible within 12 hours after sex, and no more than two doses in a 24-hour period. The women were provided with the gel on a monthly basis. The total follow-up time was 1341 person-years (mean follow-up, 18 months). The overall study retention rate was 94.8%. Additional details of the CAPRISA 004 trial have been published previously^{6,13,14}; see also the study protocol, available with the full text of this article at NEJM.org.

All women were followed up monthly with risk-reduction counseling, provision of condoms, pregnancy testing and contraception provision, clinical assessments, and safety assessments. At each monthly study visit, women were requested to return all used and unused applicators for an assessment of adherence. The frequency of use of tenofovir gel was measured by the mean number of returned empty applicators each month.

Vaginal tenofovir concentrations were measured at a single, randomly selected time point for each participant during follow-up, with the concentration serving as a biomarker of drug exposure. Concentrations were measured in undiluted aspirated cervicovaginal fluid with the use of validated, ultra-high-performance liquid chromatography–mass spectrometry.¹⁵

Caprisa 004 HSV-2 Substudy

The investigators learned of the dose-related effect of tenofovir on HSV-2 from unrelated in vitro studies when the CAPRISA 004 trial was under way and added HSV-2 as an ancillary end point to the trial before study completion. No data from the CAPRISA 004 trial played any role in the decision to add HSV-2 as an end point in the trial. Stored blood samples from the enrollment visit and last study visit were tested retrospectively (after study completion but before the un-blinding of treatment assignments) for HSV-2 antibodies, by means of an HSV-2 IgG enzyme-linked immunosorbent assay (ELISA) that uses a recombinant modified type 2 glycoprotein G (Kalon Biological); the assay was performed according to the instructions of the manufacturer. Samples with optical-density readings of less than 0.9 times the cutoff level were recorded as negative, those with values of more than 1.1 times the cutoff level were recorded as positive, and those with values in between were recorded

as equivocal. The sensitivity of this assay is reported to be 89% (95% confidence interval [CI], 83 to 94), and its specificity 85% (95% CI, 61 to 100).¹⁶

Retrospective testing of serum samples indicated that at the time of enrollment, 454 of the 889 women involved the CAPRISA 004 trial were positive for HSV-2 antibodies, 5 had equivocal serologic results, and 1 did not have results available. The remaining 429 women who were negative for HSV-2 antibodies constituted the study population for this analysis.

In the case of all women who underwent HSV-2 seroconversion as assessed by ELISA between study enrollment and exit, Western blot testing was performed to confirm the negative HSV-2 status of the sample obtained at the enrollment visit and the positive HSV-2 status of the sample obtained at the last study visit. Western blot testing was also performed on all samples with an equivocal result on ELISA. The HSV Western blot assay, which was performed at the University of Washington Clinical Virology Laboratory, uses HSV-1 and HSV-2 proteins that are separated by size to recognize type-specific antibody profiles. As an HSV-2 confirmatory test, it has a specificity and sensitivity of more than 99%.¹⁷

Study Oversight

The trial was approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal, the Protection of Human Subjects Committee of FHI 360 (a nonprofit human development organization), and the Medicines Control Council of the South African government. All authors had full access to all the data and vouch for the completeness and accuracy of the analyses presented and adherence to the study protocol.

Statistical Analysis

In planning this analysis, we calculated that with a sample size of approximately 400 women, the study would have 84% power to identify 50% efficacy of tenofovir gel after 18 months of follow-up in a population with an HSV-2 incidence rate of 12 cases per 100 person-years. The analysis was performed on an intention-to-treat basis. In the calculation of the HSV-2 incidence rate, the duration of follow-up for each woman was the period from the baseline HSV-2 test to the time of the last HSV-2 test. Gel use is contraindicated in HIV-positive women and was therefore terminated in women who became HIV-positive during the course of the trial. Thus, the follow-up period for the 22 HSV-2-negative women (9 assigned to tenofovir gel and 13 assigned to placebo gel) who became HIV-positive was excluded from the calculation of HSV-2 incidence.

For the calculation of the incidence rate, seroconversion was assumed to have occurred at the midpoint between the last HSV-2-negative result and the first HSV-2-positive result. Poisson distribution was used to calculate the confidence interval of the incidence rate. A proportional-hazards regression model with right censoring (interval-censoring methods did not meaningfully alter the results) was used to adjust for confounding variables. Fisher's exact test and the Wilcoxon two-sample test were used to compare women in the tenofovir group with those in the placebo group at baseline. Adherence categories were prespecified and were based on three strata of the mean number of empty applicators returned each month.

The ELISA results were used for the primary analysis. Sensitivity analyses were performed by including the equivocal results at study enrollment and exit as either positive or negative for HSV-2. An additional analysis was performed with the use of the Western blot results, with equivocal results excluded. The statistical analysis was performed with the use of SAS software, version 9.3 (SAS Institute).

Results

Baseline and Follow-up Characteristics

A total of 429 HSV-2–negative eligible women were followed after enrollment for an average of 18 months (range, 3 to 29). The demographic characteristics and sexual behavior at baseline were similar in the 205 women who were in the group assigned to tenofovir gel and the 224 who were in the group assigned to placebo gel (Table 1). Adjustment for potential confounding factors, such as age, parity, use or nonuse of hormonal contraception, and living or not living with a regular partner, had minimal effects on the results (Table 2). HSV-2 incidence could be assessed in 422 women; 4 women had no samples available at the end of the study for HSV-2 serologic testing, and 3 women had equivocal serologic results (Fig. 1). There were 87 seroconversions, resulting in an overall HSV-2 incidence rate of 15.5 cases per 100 person-years (95% CI, 12.4 to 19.2). A sensitivity analysis that included follow-up data on HSV-2–negative, HIV-positive women at 1 year after HIV acquisition did not materially change the HSV-2 incidence rates.

Effect of Tenofovir Gel on HSV-2 Incidence

The HSV-2 incidence rate among women who had been assigned to tenofovir gel was 10.2 cases per 100 person-years (95% CI, 6.8 to 14.7), as compared with 21.0 cases per 100 person-years (95% CI, 16.0 to 27.2) among women who had been assigned to placebo gel (incidence rate ratio, 0.49; 95% CI, 0.30 to 0.77; $P = 0.003$) (Fig. 2). The incidence rate ratio ranged from 0.48 to 0.51 in the sensitivity analyses that treated equivocal ELISA results from enrollment and exit samples as either negative or positive (Table 2).

In an as-treated post hoc analysis that was based on gel use during the trial, tenofovir gel was associated with a 71% lower risk of HSV-2 acquisition (incidence rate ratio, 0.29; 95% CI, 0.10 to 0.70) among women who used more than six gel applicators per month, a 52% lower risk (incidence rate ratio, 0.48; 95% CI, 0.18 to 1.16) among women who used four to six applicators per month, and a 27% lower risk (incidence rate ratio, 0.73; 95% CI, 0.34 to 1.55) among women who used fewer than four applicators per month.

Tenofovir Concentrations and Effectiveness

Tenofovir was detected in the cervicovaginal fluid samples of 7 of the 29 women (24%) assigned to tenofovir gel who acquired HSV-2 infection. Among the 171 women who were assigned to tenofovir gel and who remained HSV-2–negative, data on vaginal tenofovir concentrations were available for 127 women, of whom 46 (36.2%) had detectable tenofovir. In the overall population of the substudy, 156 women had data available on vaginal tenofovir concentrations. Among the 25 women with concentrations of 10,000 ng per milliliter or more, the HSV-2 incidence rate was 5.7 cases per 100 person-years, whereas

among the 103 women with no detectable vaginal tenofovir, the HSV-2 incidence rate was 15.5 cases per 100 person-years (incidence rate ratio, 0.37; 95% CI, 0.04 to 1.51; $P = 0.14$). The HSV-2 incidence rate among the remaining 28 women (those who had vaginal tenofovir concentrations of up to 10,000 ng per milliliter) was 12.3 cases per 100 person-years ($P = 0.13$ by log-rank test for trend across the three concentration strata).

Western Blot Testing

A total of 51 of the 87 ELISA-identified HSV-2 seroconversions (59%) were confirmed by Western blot testing. Of the 36 women whose seroconversion was not confirmed, 20 had equivocal results on the Western blot test and 1 had no sample available at the end of the study. Eleven of the ELISA-negative enrollment samples tested positive by Western blot assay and were regarded in the sensitivity analysis as indicating a prevalent HSV-2 infection at baseline. Four samples tested negative at both the enrollment visit and the last study visit. In addition, one sample obtained at the last study visit that had an equivocal result on ELISA was positive by Western blot assay and was therefore regarded in the sensitivity analysis as indicating a seroconversion.

The overall HSV-2 incidence rate by Western blot assay was 8.9 cases per 100 person-years (95% CI, 6.6 to 11.7), with 16 HSV-2 seroconversions among women assigned to tenofovir gel and 36 among those assigned to placebo gel. The HSV-2 incidence rate by Western blot assay among women assigned to tenofovir gel was 5.5 cases per 100 person-years (95% CI, 3.1 to 8.9), whereas the rate among women assigned to placebo gel was 12.3 cases per 100 person-years (95% CI, 8.6 to 17.0) (incidence rate ratio, 0.45; 95% CI, 0.23 to 0.82; $P = 0.005$).

Discussion

In this study in South Africa, pericoital application of tenofovir gel reduced HSV-2 acquisition by 51% among women. This estimate of effectiveness was dependent on both the efficacy of tenofovir gel and the extent to which the gel was used by women during the trial. In the absence of an effective vaccine or cure, pericoital tenofovir gel has the potential to increase the range of options for HSV-2 prevention programs, which at present promote condoms¹⁸ and circumcision.¹⁹

The anti-herpesvirus activity of tenofovir becomes evident at a concentration of approximately 10,000 to 200,000 ng per milliliter,¹¹ a range that is higher than concentrations usually attained by oral formulations of the drug.¹⁰ Hence, oral formulations of tenofovir had not been investigated for an HSV-2 indication. Since the time that the antiherpetic effects of the gel formulation of tenofovir were reported in 2010, at least three studies of oral formulations of tenofovir have generated varying results on efficacy against HSV-2. No significant effect was observed on HSV-2 shedding in HIV-coinfected patients taking daily oral tenofovir disoproxil fumarate (TDF) as part of combination antiretroviral therapy.²⁰ No significant effect on HSV-2 acquisition was seen in men who have sex with men in a trial of a daily oral TDF–emtricitabine combination for preexposure prophylaxis against HIV infection, although there may have been a reduction in genital ulceration.²¹ A preexposure prophylaxis trial of daily oral TDF and TDF–emtricitabine with high

adherence in serodiscordant couples showed a 21% reduction in HSV-2 acquisition among women and a 30% reduction overall among both men and women.²²

One explanation for the differences in protection between oral tenofovir and topical tenofovir gel is the higher vaginal drug concentrations achieved with the gel than with the oral form.¹¹ Ex vivo experiments in human lymphoid and cervicovaginal tissues have shown that the concentrations of tenofovir achieved after intravaginal gel administration⁹ have a direct antiherpetic effect by inhibiting replication of clinical isolates of HSV-2.¹¹ We found high levels of vaginal tenofovir exposure, equal to or exceeding 10,000 ng per milliliter, in some women assigned to tenofovir gel, and women with this level of exposure had a 63% higher rate of protection against HSV-2 infection than women with no detectable vaginal tenofovir. However, the precise mechanisms for the differences in observed protection levels against HSV-2 infection between tenofovir in the oral and topical formulations are not yet fully understood and require further investigation.

A significant HSV-2-protective effect from tenofovir gel was not observed in women who acquired HIV infection. This could be due to the small number of HIV-positive women in this subgroup or to low adherence to the gel, leading to similarly high incidence rates of HSV-2 infection among HIV-infected women assigned to tenofovir gel and those assigned to placebo gel. In addition to the fact that HSV-2 and HIV infections are mediated by the same set of risky sexual behaviors, there is evidence suggesting that asymptomatic HSV-2 infection (either newly acquired or preexisting) may facilitate HIV acquisition.^{23,24} A much larger and longer-term study is required to assess the way in which the effect of tenofovir gel on HSV-2 acquisition may potentially influence HIV acquisition in women.

Current HSV-2 serologic assays, including the ELISA used in this study (Kalon Biological), produce a small fraction of results with optical-density readings that are not clearly negative or positive. The 51% rate of protection observed in this trial changed little when equivocal results on ELISA were interpreted as positive or negative. With the use of Western blot testing, there were fewer confirmed HSV-2 seroconversions, principally owing to a large number of equivocal blot results. The Kalon ELISA is more likely to cross-react with antibodies than the Western blot assay¹⁷, whereas the Western blot assay may have lower specificity for a confirmatory assay in some settings.²⁵ Despite these challenges in interpretation, the Western blot data were consistent with the ELISA data in terms of the reduction in HSV-2 incidence among women assigned to tenofovir gel.

This study has several limitations. First, the trial was not originally designed to assess the effect of tenofovir gel on HSV-2 infection. Hence, randomization at enrollment was not stratified according to HSV-2 status. The subgroup of HSV-2-negative women were randomly assigned without knowledge of their HSV-2 status, and women assigned to tenofovir gel and those assigned to placebo gel had similar characteristics at baseline. Second, tenofovir concentrations at the time of HSV-2 acquisition could not be assessed. The tenofovir concentration for each woman was measured at one randomly selected study visit, and the result may or may not accurately reflect her overall exposure to tenofovir or drug concentration at the time of HSV-2 exposure. Despite this limitation, random vaginal tenofovir concentrations, which were shown in a previous study to correlate with protection

against HIV infection,¹⁵ also correlated with HSV-2 protection, especially when vaginal tenofovir concentrations were at least 10,000 ng per milliliter. An additional surrogate marker, returned empty gel applicators, was used as a proxy for overall adherence during the study. Associations between level of gel use and HSV-2 acquisition must be interpreted with caution, because the gel-use strata are based on postrandomization data. Third, HSV-2 prevalence resulted in the exclusion from this analysis of just over half the women enrolled in the CAPRISA 004 trial, thus limiting power for subgroup analyses.

Tenofovir gel could be used by women to potentially control their risk of one or more important sexually acquired viral infections. Further data on the efficacy of tenofovir gel against HSV-2 acquisition are needed, particularly among young women in Africa, who currently have among the highest rates of HSV-2 and HIV infection globally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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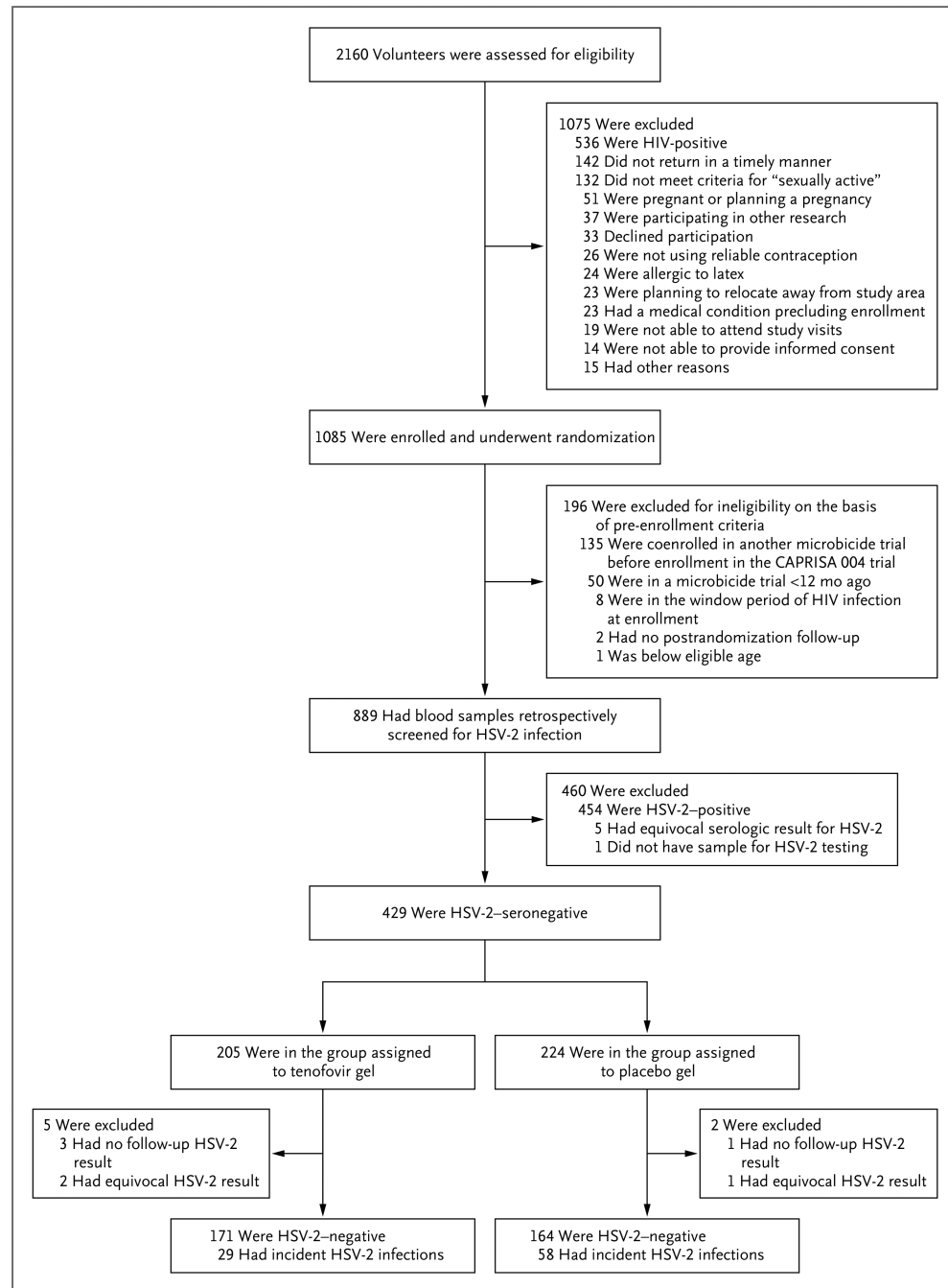


Figure 1. Screening, Enrollment, Randomization, and Follow-up of the HSV-2-Negative Participants

Positive or negative status with respect to herpes simplex virus type 2 (HSV-2) infection was determined retrospectively from stored samples. CAPRISA denotes the Centre for the AIDS Programme of Research in South Africa.

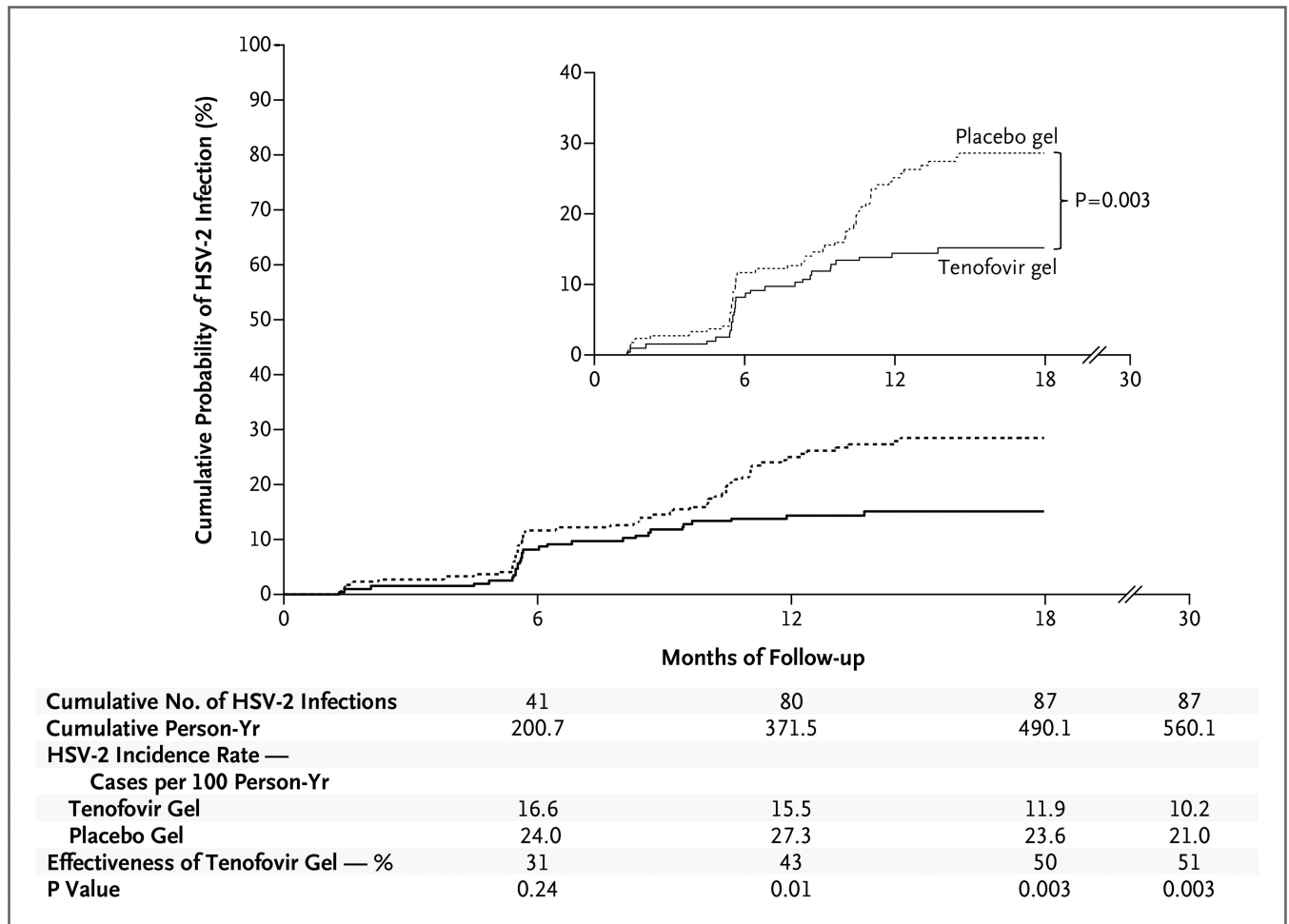


Figure 2. Kaplan–Meier Estimates of Cumulative Probability of HSV-2 Infection

HSV-2 status was determined by enzyme-linked immunosorbent assay. Data are shown for the first 18 months of follow-up; there were no HSV-2 infections estimated to have occurred in the small number of women who were followed beyond 18 months. The inset shows the same data on an enlarged y axis.

Table 1

Sociodemographic and Behavioral Characteristics of Women with Negative Serologic Testing for HSV-2 at Enrollment, According to Study-Group Assignment.*

Variable	Tenofovir Gel (N = 205)	Placebo Gel (N = 224)
Sociodemographic characteristic		
Age — yr	22.2±3.6	21.6±3.4
Parity	0.7±1.0	0.7±0.8
High-school education or more — no. (%)	92 (44.9)	98 (43.8)
Income per month <1000 rands — no./total no. (%) [†]	167/179 (93.3)	173/185 (93.5)
Using hormonal contraception — no. (%)	202 (98.5)	224 (100)
Stable partner — no. (%) [‡]	202 (98.5)	222 (99.1)
Living with regular partner — no. (%) [§]	18 (8.8)	9 (4.0)
Sexual behavior		
Age at first sexual experience — yr	17.5±2.1	17.4±1.8
Age of oldest partner in past 30 days — yr	25.2±4.3	24.8±4.2
No. of lifetime sex partners	2.2±2.1	3.0±12.1
No. of sex acts in past 30 days	8.5±8.2	7.5±6.8
New sex partner in past 30 days — no. (%)	1 (0.5)	3 (1.3)
Knows sex partner had other sex partners in past 30 days — no. (%)	35 (17.1)	41 (18.3)
Knows sex partner had HIV test in past 30 days — no. (%)	3 (1.5)	7 (3.1)
Reports alcohol consumption by partner and self before sex in general — no. (%)	7 (3.4)	7 (3.1)
Reports alcohol consumption by partner or self before the last time they had sex — no. (%)	51 (24.9)	59 (26.3)
Reports always using condom during sex — no. (%)	61 (29.8)	63 (28.1)
Location of study site — no. of participants (%)		
Urban	57 (27.8)	57 (25.4)
Rural	148 (72.2)	167 (74.6)

* Plus-minus values are means ±SD. There were no significant differences between the two groups, except for the difference in mean parity (P = 0.01) and in living with a regular partner (P = 0.03). HIV denotes human immunodeficiency virus, and HSV-2 herpes simplex virus type 2.

[†] A value of 1000 South African rands is equal to approximately 80 U.S. dollars.

[‡] A stable partner was defined as someone with whom the woman has a regular sexual relationship.

[§] A regular partner included a husband or a stable partner.

Table 2

Incidence of HSV-2 Infection and Effectiveness of Tenofovir Gel for HSV-2 Prevention, According to Study Site, Equivocal HSV-2 Results, and HIV Status.*

Variable	Tenofovir Gel			Placebo Gel			Incidence Rate Ratio (95% CI)	Effectiveness of Tenofovir Gel	P Value [†]
	HSV-2 Infection no./total no.	Person-Yr	Incidence Rate cases per 100 person-yr (95% CI)	HSV-2 Infection no./total no.	Person-Yr	Incidence Rate cases per 100 person-yr (95% CI)			
Overall [‡]	29/200	284.0	10.2 (6.8–14.7)	58/222	276.0	21.0 (16.0–27.2)	0.49 (0.30–0.77) [§]	51	0.003
Study site [‡]									
Urban	8/56	77.4	10.3 (4.5–20.4)	19/57	65.0	29.3 (17.6–45.7)	0.35 (0.13–0.85)	65	0.02
Rural	21/144	206.6	10.2 (6.3–15.5)	39/165	211.0	18.5 (13.1–25.3)	0.55 (0.31–0.96)	45	0.04
Assignment of equivocal results of HSV-2 ELISA									
Equivocal results treated as negative	29/205	293.1	9.9 (6.6–14.2)	58/225	279.0	20.8 (15.8–26.9)	0.48 (0.29–0.76)	52	0.002
Equivocal results treated as positive	31/202	288.0	10.8 (7.3–15.3)	59/223	277.2	21.3 (16.2–27.5)	0.51 (0.32–0.79)	49	0.004
HIV infection status [‡]									
Not infected	20/182	267.8	7.5 (4.6–11.5)	47/198	259.1	18.1 (13.3–24.1)	0.41 (0.23–0.71)	59	0.001
Infected	9/18	16.3	55.2 (25.2–104.8)	11/24	16.8	65.3 (32.6–116.9)	0.85 (0.31–2.24)	15	0.90

* HSV-2 infection was determined by an enzyme-linked immunosorbent assay (ELISA).

[†] P values were calculated with the log-rank test.

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[‡] Equivocal HSV-2 results were excluded from analysis.

[§] Analysis of this comparison with a Cox proportional-hazards model generated a hazard ratio of 0.52 (95% CI, 0.33 to 0.81). After adjustment for potential confounders, including living or not living with a regular partner, age, parity, and the use or nonuse of hormonal contraception, the hazard ratio was 0.54 (95% CI, 0.35 to 0.85).